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Epigenetic drug screen of natural killer cells identified compounds controlling immune training and tolerance

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Abstract

Natural killer (NK) cells are cytotoxic innate lymphocytes that provide defense against pathogens and malignancy. New evidence identified that NK cells are capable of memory-like immune responses in certain settings. This innate immune memory is thought to be imprinted through epigenetic modifications. However, precise epigenic pathways or strategies to modulate long-term NK cell activity are not well defined. In this study, we performed a screen of an epigenetic library containing 160 drug compounds with well-characterized epigenetic regulatory mechanisms, to identify drugs and pathways that could train or tolerize NK cell activation. One compound that we identified was the H3K27 methyltransferase Ezh2, which regulated NK cell lineage commitment from bone marrow hematopoietic stem cells and altered the phenotype of differentiated NK cells to increased cytotoxicity. This outcome provides an option to train or induce stronger cytotoxic effects on NK cells. In contrast, another compound we identified was the histone deacetylase inhibitor, Givinostat, which did not alter NK cell commitment from bone marrow stem cells, but significantly decreased NK cell function in the periphery resulting in a more tolerant innate immune state to secondary NK cell stimulation. Identification and exploration of these epigenome altering drugs may offer further insight into downstream pathways of NK cell function or provide novel therapies for tolerizing or priming innate immune responses.

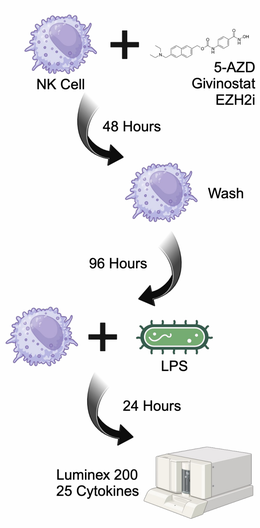
Introduction

- NK cells play a vital role in immune surveillance against infections and unhealthy cell types.
- Traditionally considered part of innate immunity, NK cells also exhibit adaptive traits.
- Therapeutics and signaling molecules have been identified to be able to induce tolerance of immune cells[2], decrease inflammation and activation[3], or train cells to produce elevated inflammation and increased activation of immune cells[4].
- Transcription factors involved in regulating NK differentiation and function have been identified[1], however, the genetic and epigenetic regulatory networks of NK function still require more exploration.
- Epigenetic factors such as histone modification through methylation or acetylation, and DNA methylation may contribute to NK cell abilities and function[5].
- Using small molecular compunds that target epigenetic regulation could identify functional targets to control the state of the innate immune system

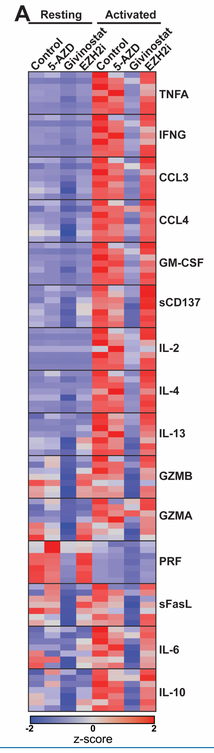
1. Bradley et al 2018, *Cell*, **2**. Foster et al 2007, *Nature*, **3**. Ripamonti et al 2022, *Front. Immunol*, **4**. Luetke-Eversloh et al 2014, *PLoS Pathogens* **5**. Li et al 2017, *Oncotarget*

Our goal is to better understand the epigenetic mechanisms behind tolerance and training with hopes to be able to harness those tools for targeting diseases and controlling aggressive immune phenotypes.

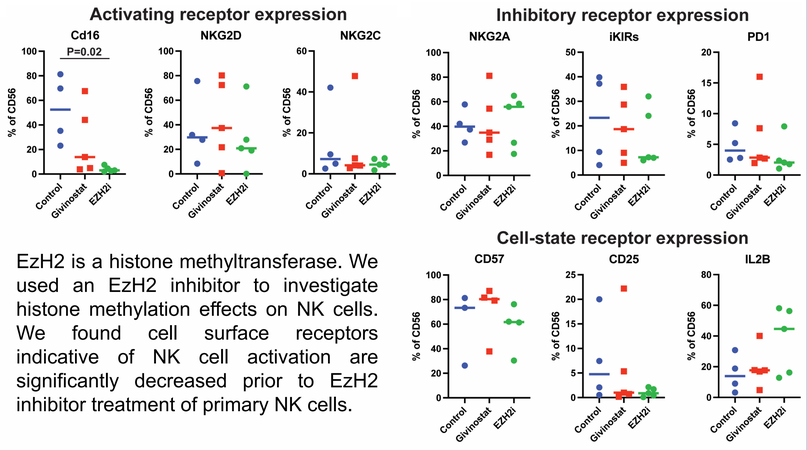
Primary Givinostat treatment produces tolerance profile of primary NK cells when activated with secondary LPS



Givinostat is an FDA approved histone deacetylase (HDAC) inhibitor therapeutic for Duchenne Muscular Dystrophy (DMD).
NK cells were treated for 48 hours with Givinostat to investigate the effect of HDAC inhibitors.
15 pro-inflammatory cytokines secreted by primary NK cells were decreased after treatment with Givinostat compared to control after secondary activation with LPS.

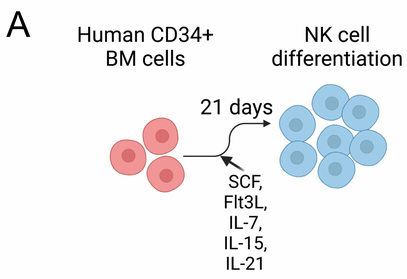


EzH2 Inhibitor decreases activating receptor expression

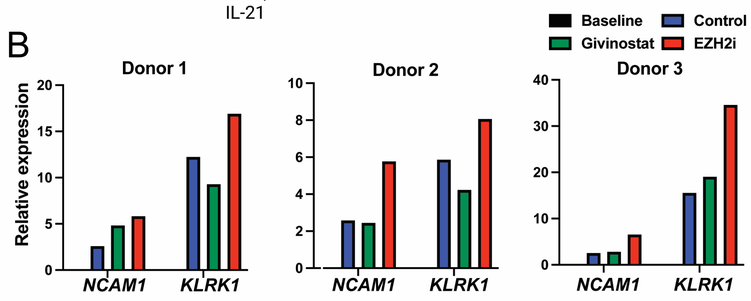


EzH2 is a histone methyltransferase. We used an EzH2 inhibitor to investigate histone methylation effects on NK cells. We found cell surface receptors indicative of NK cell activation are significantly decreased prior to EzH2 inhibitor treatment of primary NK cells.

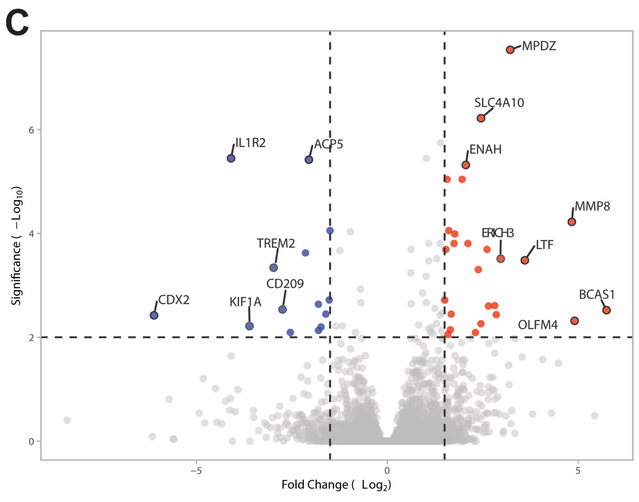
EzH2 Inhibitor treatment of CD34+ Stem Cells induces NK Cell Differentiation with pro-inflammatory cell type



A) Flow Cytometry: 3 unique donor primary CD34+ bone marrow cells were treated with mock, Givinostat, or EzH2 inhibitor prior to 21 days of selective cytokines to induce NK cell differentiation

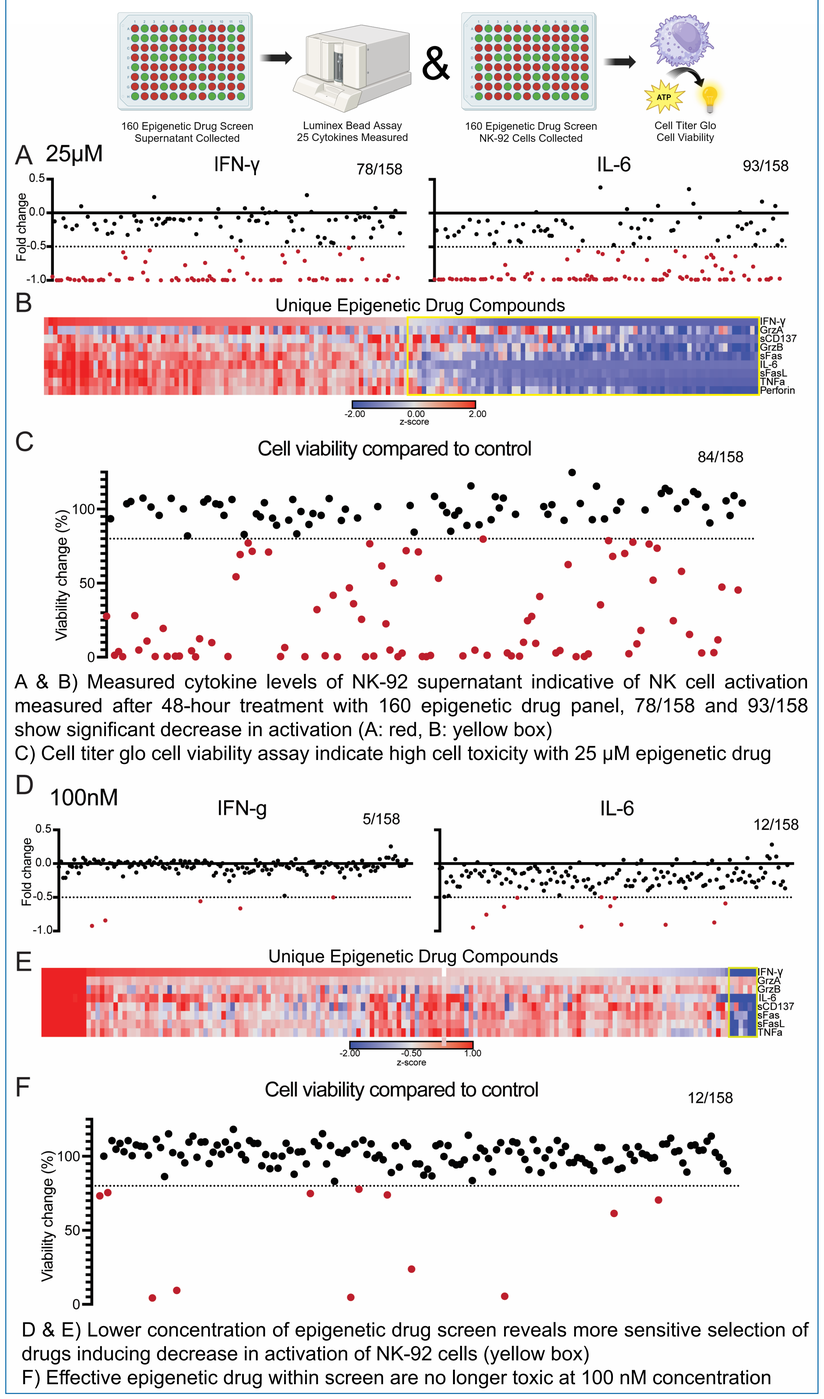


B) RNA Sequencing: Expression of NCAM1 and KLRK1, markers of NK cell activation, are more highly expressed in NK cells differentiated from CD34+ bone marrow cells treated with EzH2 Inhibitor in all 3 donor cells compared to control.



Volcano plot of genes representing activation of NK cells indicate EzH2 inhibition prior to differentiation of CD34+ stem cells cause an elevated activation profile of NK Cells indicating a pro-inflammatory cellular profile compared to control NK Cells.

Epigenetic Drug Screen identifies drug types causing decrease in NK-92 activation cytokines



A & B) Measured cytokine levels of NK-92 supernatant indicative of NK cell activation measured after 48-hour treatment with 160 epigenetic drug panel, 78/158 and 93/158 show significant decrease in activation (A: red, B: yellow box)
C) Cell titer glo cell viability assay indicate high cell toxicity with 25 μM epigenetic drug

D & E) Lower concentration of epigenetic drug screen reveals more sensitive selection of drugs inducing decrease in activation of NK-92 cells (yellow box)
F) Effective epigenetic drug within screen are no longer toxic at 100 nM concentration

Conclusions and Future Directions

1. Givinostat induce tolerance for primary NK cells, lowering inflammation with secondary infections
2. EzH2 Inhibitor decreases activation natural killer cell surface receptors
3. Stem cell exposure to EzH2 inhibitor induces a pro-inflammatory and elevated activation profile of differentiated NK cells. Provides avenue for longitudinal training of NK cells for more aggressive and active profile
4. Identified 2 families of epigenetic drugs with low toxicity able to induce decreased NK-92 cells from secreting cytokines indicative of activation and inflammation

Future directions include identifying if these newly identified epigenetic drug families can induce tolerance, investigating CD34+ stem cell treatment and the resulting altered differentiation inflammatory profiles of NK cells, and allow us to utilize these newly identified tools to better control NK cell regulation and activation.

Acknowledgements

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